2011

# the Relationship between Vitamin D Statuses and Young Adult Women Asthma

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# THE RELATIONSHIP BETWEEN VITAMIN D STATUSES AND YOUNG ADULT WOMEN ASTHMA

A Thesis Presented

by

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Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

February 2011

Nutrition

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# ABSTRACT

THE RELATIONSHIP BETWEEN VITAMIN D STATUSES
AND YOUNG ADULT WOMEN ASTHMA

February, 2010

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Although maternal vitamin D status has been linked to asthma in offspring, the relationship between vitamin D status and asthma in adults still remains unclear. The current study assessed the relationship between measures of vitamin D status and self-reported asthma/wheeze in 186 healthy women aged 18-30 years. Although the risk of asthma/wheeze symptoms was three-times higher among women with low dietary vitamin D intake (<200 IU/day) than in those with higher vitamin D intake, suboptimal serum levels of 25(OH)D ( <70 nmol/L) were associated with a 48% lower risk of asthma/wheeze than “optimal” serum levels. These contradictory effects underscore the poor correlation between dietary vitamin D intake and serum vitamin levels and suggest that other components in vitamin D-rich foods may be protective. Alternatively, women with higher serum vitamin D levels may have spent more time outdoors, increasing their exposure to asthma triggers. This study also identified predictors of serum 25 (OH) D in this sample. In addition to total dietary vitamin D (r= 0.2; p=0.03), intake of cold cereal (p=0.02) also significantly predicted serum 25(OH)D levels. Among non-dietary factors,

month of blood draw ($p=0.05$) and oral contraceptive use ($p<0.0001$) were positive predictors of serum 25(OH) D; sunscreen use ($p=0.04$) was a negative predictor. After adjusting for covariates, oral contraceptive use was associated with 25(OH)D levels that were on average 24 nmol/L greater than those observed in women who did not use oral contraceptives. Additional prospective studies are needed to further evaluate the relationship between vitamin D status and asthma.

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# CHAPTER 1

# INTRODUCTION

Although the role of vitamin D in bone health is well known, recent studies have described new, nonskeletal roles for vitamin D in human health, including a role in preventing chronic diseases such as cardiovascular disease, diabetes and cancer. (Wang, 2008; Mohr, 2008; Holick, 2004a; Holick, 2004b)

In addition to these chronic diseases, vitamin D status may also be linked to asthma, although this area of research is relatively new (Black, 2005, Burns 2006, Camargo, 2007). Asthma is a common chronic lung disease that involves inflammation of the pulmonary airways and bronchial hyper-responsiveness, which manifests as lower airway obstruction (Expert Panel Report 3, 2007). Asthma is a common disease worldwide and affects 22 million people in the United States alone. Although the overall mortality rate for asthma has declined and asthma-related hospitalizations have remained constant since 1995 (Centers for Disease Control and Prevention asthma surveillance data; Moorman 2007), asthma nonetheless contributes substantially to morbidity. Children and African Americans are especially affected by asthma; the prevalence of asthma is twice as high in these groups as in other groups (Centers for Disease Control and Prevention asthma surveillance data; Moorman 2007). The cost of treating asthma continues to be a large burden in health care costs for both adults and children.

The prevalence of asthma increased throughout the late 1980s in developed countries, leading to many hypotheses that attempted to explain this increase. One hypothesis, the “hygiene hypothesis,” suggested that declining family size reduced exposure to infectious pathogens in early childhood, and higher standards of cleanliness, compromise development of immune function which then increases susceptibility to allergic diseases (Strachan, 1989), including asthma. Although this phenomenon may account for some of the increase in asthma prevalence, it does not completely explain the asthma epidemic. Other factors, including urban poverty (Platts-Mills, 2001), obesity (Ford, 2005) and dietary components also appear to play a role (Raviv, 2010).

Litonjua and Weiss (2007) recently offered a new hypothesis to explain the asthma epidemic. They hypothesized that vitamin D status influences asthma risk, although they had direct evidence of a relationship only among pregnant women and their offspring. Whether vitamin D status is associated with adult asthma remains unclear. The main goal of the current project is to assess the relationship between vitamin D status and asthma in young adult women.

# CHAPTER 2

# ASTHMA

The term "asthma" comes from the ancient Greek language; it means “to breathe hard." Current medical terminology defines this condition as the reversible obstructive airway disease (ROAD). “Asthma is a chronic lung air way disorder, characterized by episodic and reversible airflow obstruction, bronchial hyper- responsiveness and underlying inflammation” (Expert Panel Report 3, 2007).The symptoms are highly variable both among and within patients, ranging from mild illness to death. Clinical manifestations include wheezing, coughing, and shortness of breath (Expert Panel Report 3, 2007). Asthma can resolve spontaneously or require treatment.

According to the Center for Disease Control and Prevention data, from 1980 to 2004, the prevalence of asthma continued increasing in the United States. Asthma increased in prevalence during 1980-1996, but since 1997 no discernable change has been identified in asthma attack estimates. The overall prevalence of asthma in the general population was about 7% in 2006. For the three-year period 2001-2003, 20 million people annually had diagnosed asthma in the United States. Of these, approximately 6.2 million were children (aged <18 years) and 13.8 million were adults. Approximately 2 million persons were aged ≥65 years. Overall mortality rates have declined and hospitalizations have remained constant since 1995 due to advances in diagnosis and treatment. The incidence of asthma is greater in children of all races as

well as in African-Americans. Among children under the age of 10, asthma affects twice as many boys as girls. But with increasing age, women are more likely to have asthma compared to men (Stachan, 1996). Half of all cases first occur in children younger than age 10. There are no clear explanations to explain the differentiations occur in age and gender.

No single instrument can be used to identify asthma with certainty. Asthma is a clinical diagnosis made by physicians on the basis of a patient's medical history, physical examination, assessment of reversibility of airway obstruction and exclusion of alternative diagnoses that can mimic asthma (EPR 3, 2007). Since each physician has a different diagnosis level, this causes difficulty in asthma research and data collection. Sometimes changes in asthma prevalence can be at least partly attributable to subjective changes in diagnostic labeling.

## The Pathophysiology of Asthma

The pathophysiology of asthma is complex and involves three main characteristics: inflammation, reversible airway obstruction and bronchial hyper-responsiveness. (Busse, 2001)

### Inflammation

Inflammation plays the central role in development and expression of asthma. Inflammation not only causes the airway obstruction, but also contributes to airway hyper-responsiveness, which enhances susceptibility to bronchospasm. The model that infections could trigger and aggravate asthma in already sensitized individuals has been well established. (Cohn, 2004).

Inflammation in asthma is the result of a complex process that involves the participation of different cell types in various tissues comprising the respiratory tract. The cellular profile and the response of structural cells in asthma are quite consistent regardless of differences in asthma presentation. Some of the principal cells identified in airway inflammation include mast cells, eosinophils, epithelial cells, macrophages, and activated T lymphocytes. T lymphocytes play the most important role in the regulation of airway inflammation because they release numerous cytokines that regulate immune function (Roberto, 2006; Busse, 1996).

The pattern of inflammation with viral infections and its effect on asthma are distinct from that associated with allergen activation. Exposure to allergens causes airway eosinophilic responses that are the end step controlled by the T cell immune response, whereas viral infections elicit neutrophilic responses (Abu-Harb, 1999; Pizzichini, 1998)

The presence and absence of infections may explain variation over time in susceptibility to asthma in an individual. The chronic inflammation causing airway remodeling in some patients may be associated with permanent alterations in the airway structure (Buss, 2001). This can lead to more severe asthma later in life.

**Bronchial hyperresponsiveness**

Airway hyper-responsiveness is an exaggerated response to both exogenous and endogenous stimuli. It leads to clinical symptoms, airway narrowing and subsequent interference with airflow. The mechanisms involved include direct stimulation of airway smooth muscle and indirect stimulation by physiologically active substances from mediator-secreting cells, such as mast cells (Hargreave, 1981). Measurements of airway

responsiveness appear to reflect the activity and severity of asthma at the time of measurement rather than to define the prevalence of the illness over a specific period (EPR3, 2007).

**Reversible airway obstruction**

Airflow obstruction can be caused by a variety of changes, including acute bronchoconstriction, airway edema, airway hyper-responsiveness, and airway remodeling (Finucane, 1985). Acute bronchoconstriction is the consequence of immunoglobulin E–dependent mediator release following exposure to aeroallergens and is the primary component of the early asthmatic response. Airway edema occurs 6-24 hours following an allergen challenge and is referred to as the late asthmatic response. Airway hyper-responsiveness involves narrowing of the airway when exposed to stimuli, such as food and medicine. Airway remodeling is associated with structural changes due to long-standing inflammation and may profoundly affect airway obstruction. (Holgate, 2000)

## Etiology (Pathogenesis)

Although the pathophysiology of asthma is fairly well understood, the exact etiology of asthma is still not known. For many patients, the disease has its roots in infancy. Both host factor and environmental factors influence the development and exacerbation of asthma. Although environmental factors impact asthmatic conditions in children, susceptibility to asthma is influenced by genetic factors as well (Holgate, 1999a).

## Host Factors

**Genetics** Many genes have been found that either are involved in or linked to the presence of asthma. Asthma is known as a "complex" heritable disease. This means that there are a number of genes that contribute toward a person's susceptibility to a disease. Researchers cannot find one exact gene that results in asthma; rather, genes on chromosomes 5, 6, 11, 12, and 14 have all been implicated (Holgate, 1999a), especially for allergen-induced asthma. According to the latest Expert Panel Report (EPR) in 2007, the genetic predisposition for the development of an IgE-mediated response to common aeroallergens is the strongest identifiable predisposing factor for developing asthma."

Genetics may also contribute to asthma by influencing genes involved in immunity. Airway inflammation in asthma may represent a loss of normal balance between two populations of Th lymphocytes, Th1 and Th2. Th1 cells produce IL-2 and IFN-ɣ, which are critical in cellular defense mechanisms in response to infection. Th2 cells, in contrast, generate a family of cytokines that can mediate allergic inflammation, and asthma is characterized by a shift toward cytokine-like disease. Therefore this cytokine imbalance toward Th2 is caused either by overexpression of Th2-type cytokines or underexpression of Th1-type cytokines (Sundeep, 2001). This genetic background will promote the production of antibody to key environmental antigens, and then increase an individual’s susceptibility to asthma (Larche, 2003).

**Sex** In early life, the prevalence of asthma is higher in boys, with a male-to-female ratio of 2:1 until adolescence. After that time, asthma prevalence is greater in females, and the majority of adult-onset cases diagnosed in persons who are older than 40 years occur in women (Fagan,2001).

**Race** Asthma occurs in people of all races worldwide. In the United States, asthma prevalence as well as its morbidity and mortality are higher in blacks than in whites (ERP 3, 2007).

**Environmental factors**

Three environmental factors appear to be involved in the development and the persistence of asthma. They do not work separately but interactively in the eventual development of asthma.

**Allergens** House dust mites, animal allergens (especially cat and dog), cockroach allergens, and fungi are the most commonly reported allergens. Sensitization and frequency of exposure to allergens are important factors in the development of asthma in children (Busse, 2001; McConnell, 2002)).

**Respiratory infections** Viral infections can exert influence on both the development and the severity of asthma. They are an important cause of acute wheezing in infancy, and viruses are detected in most exacerbations of asthma throughout childhood. Furthermore, infants who develop severe viral respiratory infections are more likely to have asthma later in childhood. (Pelaia,2006; Gern, 2002 ).

**Other environmental exposures** Research has concluded that maternal smoking can contribute to asthma or other impairment of infant lung function, even before the child is born. Continued exposure to cigarette smoking can irritate the respiratory tract and make infants and children particularly vulnerable to allergic asthma (Ulrik, 2001).

It has been suggested that changes in dietary habits in recent years may influence asthma development. Diets that are deficient in antioxidants such as vitamins C and E may explain some of the rise in asthma and allergy prevalence (Weiss, 1997; Romieu, 2001).

Epidemiologic studies have shown that the prevalence of obesity and that of asthma are increasing together. The prevalence of asthma is higher in obese than lean individuals (Ford, 2005). The reason for this association is still unknown. The possible suggested mechanisms include reduced chest wall by obesity or immune function change by obesity–related hormones (Shore, 2008).

Exposure to air pollution has also been associated with an increased risk for onset of asthma, although the association is not as clear as the relationship between allergens and respiratory infection (Peden, 2001).

## Classification of Asthma

Over the years, asthma has been classified using different classification schemes, each of which has its own advantages and disadvantages. The most common classification divides asthma into two general categories: extrinsic (allergic) asthma and intrinsic (non-allergic) asthma, depending on the types of stimuli that trigger attacks (Busse, 1996).

**Extrinsic asthma (allergic asthma)** Extrinsic asthma is a result of an antigen\antibody reaction involving mast cells in the respiratory tract. The allergen is an antigen that elicits IgE antibodies that are normally used to attack parasitic worms. This allergen and antibody reaction causes the release of inflammatory mediators from mast cells that elicit the clinical response associated with an asthma attack. Most childhood asthma is considered an allergic type of asthma (Busse, 1996).

In allergic individuals, injection of protein just under the skin triggers an immediate skin reaction, such as swelling or redness. An individual that has this type of reaction is described as "atopic". Often extrinsic asthma is also called atopic or "allergic" asthma (Holgate, 1999). People with allergic asthma and their families frequently have other allergy-related problems. A positive family history of this type of reaction frequently exists in patients with allergic asthma. Allergic asthma usually responds quite well to the use of inhaled steroids, which suppress the immune system, particularly in the lungs where the reaction is created (Holgate, 1999b).

Infants and young children have a high response to food allergies that produce wheeze, but this response becomes much less frequent by the time the child reaches age 10. From about 4 years of age, children develop allergies to inhaled substances,

and this response may stay with the person for a lifetime. After young adulthood, allergic reactions often decrease and so does the asthmatic response (Holgate, 1999b).

**Intrinsic Asthma** Intrinsic asthma is not allergy-related. In fact, it can be caused by substances that are not proteins. Possible causes of intrinsic asthma symptoms include respiratory irritants such as perfumes, cleaning agents, fumes, smoke and cold air, upper respiratory infections, and gastroesophageal reflux (GERD) (Holgate, 1999b)

Antibodies are not produced and antigen skin testing shows no reaction. With intrinsic asthma, an asthmatics' airways are unusually sensitive or "hyper-reactive," and an attack may be triggered by an irritation to the nerves or muscles in the airways. Despite the differences in triggers, both intrinsic and extrinsic asthma present with similar symptoms. Intrinsic asthma is not likely to develop in children; its typical onset occurs after age 40 (Buss, 2001). Prevention and treatment of intrinsic asthma are not easy. Since it is difficult to identify the asthma triggers, avoiding those triggers can be virtually impossible.

## Symptoms and Diagnosis of Asthma

The common symptoms of an asthma episode include wheezing, chest tightness, coughing, and shortness of breath, although these symptoms are present only some of the time (Holgate, 1999b). Asthma is a clinical diagnosis made by physicians on the basis of a patient’s medical history, physical examination, assessment of reversibility of airway obstruction and exclusion of alternative diagnoses that mimic asthma.

In order to help treat asthma more effectively, in 1997 the National Heart, Lung and Blood Institute identified four levels of asthma severity based on the frequency of symptoms and exacerbations, effects on activity level, and lung function study results: mild intermittent, mild persistent, moderate persistent, and severe persistent. This classification is still used by most physicians.

***Guideline Similarities***

**Asthma severity and treatment**

| | | | For children >5 yr who can use spirometer or peak flow meter | |
|---|---|---|---|---|
| | Days with Symptoms | Nights with Symptoms | $FEV_1$ (% pred.) PEF (% pred.) | PEF Variability |
| 4 **Severe Persistent** | Continual | Frequent | ≤60% | >30% |
| 3 **Moderate Persistent** | Daily | >4/month | >60%-<80% | >30% |
| 2 **Mild Persistent** | >2/week | >2/month | ≥80% | 20-30% |
| 1 **Mild Intermittent** | ≤2/week | ≤2/month | ≥80% | <20% |

# CHAPTER 3

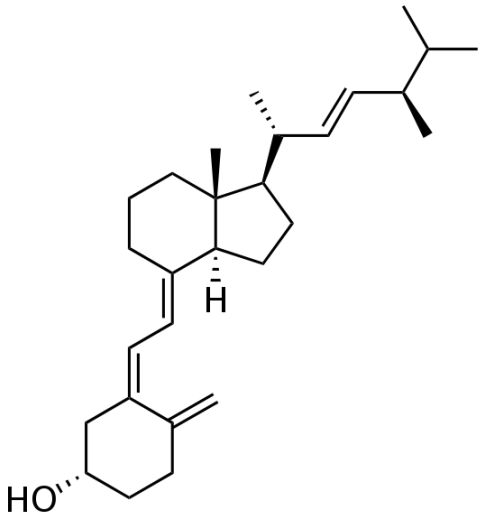
# VITAMIN D

## Introduction

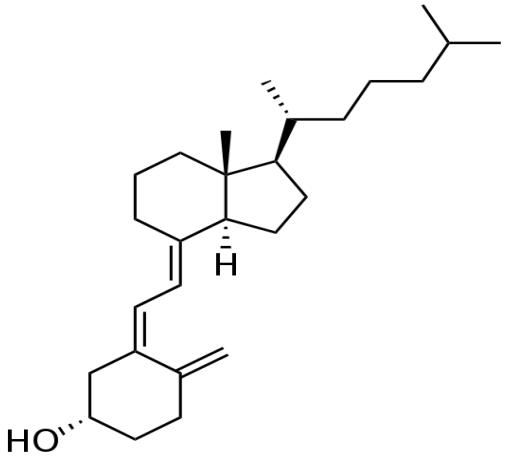
Vitamin D was first discovered in 1920 by Mellanby. It was originally described as a “vitamin” that is essential for normal skeletal development and maintenance of calcium homeostasis in the body. With further research, it was discovered that vitamin D does not strictly meet the definition of a vitamin since it can be synthesized in the body from exposure to sunlight. Instead, vitamin D behaves more like a steroid hormone than a vitamin, and its effects on health and disease depend on this hormonal action.

Vitamin D can be obtained from dietary sources or can be synthesized in the body. Dietary vitamin D is available in two forms: vitamin $D_2$ (ergocalciferol) from plant sources and vitamin $D_3$ (cholecalciferol) from animal sources, both of which are collectively called vitamin D. However, dietary sources of vitamin D account for a very small amount of the total vitamin D in the body since few foods naturally contain vitamin D. Most vitamin D circulating in the bloodstream is synthesized in the skin from 7-dehydrocholesterol (7-DHC) when it is exposed to ultraviolet B light. Vitamin $D_2$ and vitamin $D_3$ have only a slight structural difference in their side chain. Neither vitamin form has biological activity; both need to be activated to exert vitamin D-related effects.

**Figure 3.1 Structures of vitamin D2 and $D_3$**




Vitamin $D_2$ (ergocalciferol)




Vitamin $D_3$ (cholecalciferol)

## Sources of Vitamin D

### Diet

As mentioned above, vitamin D is derived from two main sources: the diet and cutaneous synthesis. Fatty fish and cod liver oil are among the few foods that naturally contain vitamin D. In the U.S., milk and some dairy foods, juices and cereals are fortified with vitamin D, although fortification is not common in other parts of the world. Vitamin D is also available in supplements, either alone or with calcium and other nutrients (Holick, 2008). Table 3.1 lists the vitamin D content in various foods.

**Table 3.1 Vitamin D Content of Selected Foods and Supplements**

| Source | Vitamin D content (IU) |
|---|---|
| Natural source | |
| Salmon Fresh, wild( 3.5oz) | 600-1000 |
| Fresh, farmed( 3.5oz) | 100-200 |
| Canned( 3.5oz) | 300-600 |
| Mackerel, canned( 3.5 oz) | 250 |
| Tuna, canned (3.6 oz) | 230 |
| Cod liver oil ( 1 tsp) | 400-1000 |
| Shiitake mushrooms Fresh (3.5oz) | 100 |
| Sun-dried (3.5oz) | 1600 |
| Fortified foods | |
| Fortified milk ( 1 cup) | 100 |
| Fortified orange juice ( 1 cup) | 100 |
| Fortified yogurts( 1 cup) | 100 |
| Fortified breakfast cereals ( 1 cup) | 100 |
| Fortified butter ( 3.5oz) | 50 |
| Fortified cheese ( 3.5oz) | 100 |
| Supplements | |
| Prescription vitamin $D_2$ | 50,000 |
| Prescription drisdol liquid supplements | 8000 |
| Vitamin $D_3$ | 400,900,1000,2000 |
| Multivitamin | 400 |

40 IU=1 ug vitamin $D_3$

**Cutaneous synthesis of vitamin D**

Because few foods contain vitamin D, most of the vitamin in circulation is derived from cutaneous synthesis. Humans (and most animals) can synthesize vitamin D in the skin when it is exposed to ultraviolet radiation. Solar ultraviolet B radiation (wavelength between 290 to 315 nm) causes 7-dehydrocholesterol to isomerize to previtamin $D_3$, which at body temperature thermally isomerizes to vitamin $D_3$. The whole isomerization procedure may require one hour to days to produce vitamin $D_3$. In ideal conditions, nearly 3000 IU of vitamin D can be synthesized cutaneously following an average of 5 to 10 minutes of UVB exposure of the arms and legs. Excess exposure to sunlight will not cause toxicity since solar UV radiation will degrade the extra previtamin $D_3$ and vitamin $D_3$ into inactive photoproducts (Holick, 2004).

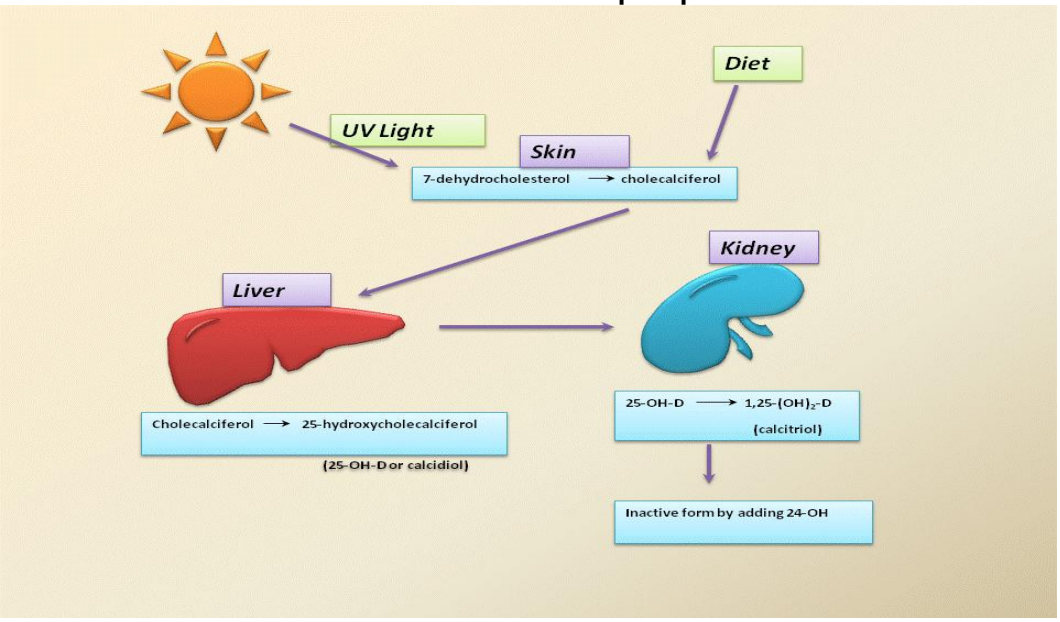
Numerous factors influence cutaneous vitamin D synthesis. First, only a narrow range of wavelengths (290-315 nm ultraviolet B) can stimulate cutaneous synthesis. Consequently, any geographical area above a latitude of 37 degrees in the northern hemisphere or below 37 degrees in the southern hemisphere does not receive the appropriate wavelength of ultraviolet B during the winter months ( such as November through February in the Boston area ), since most UV-B is absorbed by the ozone layer at that period. Therefore, vitamin D is not synthesized during winter. Second, public health messages concerning the association between sun exposure and skin cancer has led to widespread use of sunscreens. However, any sunscreen with a skin protection factor (SPF) of 8 or more inhibits vitamin D synthesis by absorbing UV-B radiation. Melanin in skin also inhibits cutaneous vitamin D synthesis, and dark skinned persons need up to 10 times as much sun exposure as lightly pigmented persons to synthesize an equivalent amount of vitamin $D_3$. Third, clothing can also impede vitamin D synthesis,

and women in some parts of the world who wear veils and other concealing clothing for cultural reasons have a limited ability to obtain vitamin D by cutaneous synthesis ( Holick, 2007).

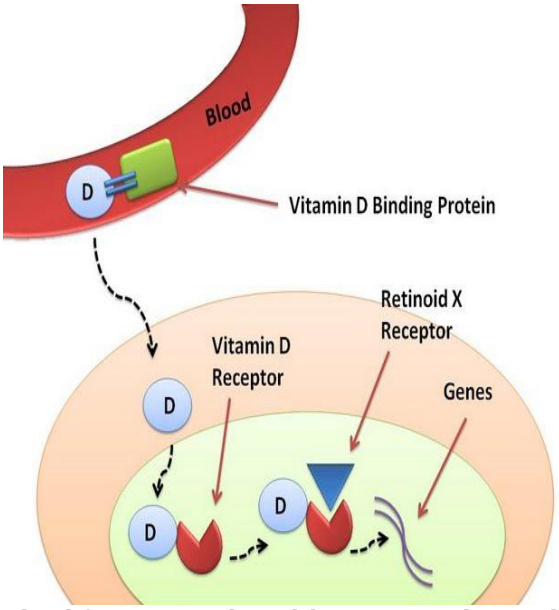
## Metabolism of Vitamin D

Vitamin $D_3$ and Vitamin $D_2$ from dietary sources or cutaneous synthesis are transported together to the venous circulation by the lymphatic system. In the circulation, it binds to the vitamin D binding protein (DBP), which transports the vitamin to target cells for metabolism. In the liver, vitamin D is converted by the enzyme 25-hydroxylase to 25-hydroxyvitamin $D_3$ [25(OH)D], which is the main circulating form of the vitamin D. However, 25(OH)D remains biologically inactive until it is further hydroxylated to 1,25(OH) $_2D_3$ (also known as calcitriol), the active form of the vitamin, by 1α–hydroxylase, which is found primarily in the kidney . Calcitriol then generates biological responses in over 30 target tissues. Activation of vitamin D is outlined in Figure 3.2

**Figure 3.2 Synthesis of calcitriol**
**Cited from Mineral mechanism in nephropal .com**



Calcitriol works as a switch that can turn genes “on” or “off.” The vitamin D receptor (VDR) works as a transcription factor inside the cell. Once bound by calcitriol, the activated VDR binds to a companion protein, the retinoid-x receptor (RXR), forming a heterodimer that binds to a specific region of the cell’s DNA near to the target gene. Attachment of the heterodimer to the DNA induces the cellular machinery to begin transcribing the nearby gene into mRNA that the cell will translate into a protein. By causing a cell to make a particular protein, calcitrol alters cellular function, and this ability to trigger gene activity in different cells is the basis of vitamin D‘s physiological effect (Norman et al, 2001; Figure 3.2)




At least 1000 different genes are believed to be regulated by 1,25(OH) $_2D_3$, including several involved in vitamin D’s traditional role in calcium and bone metabolism and other newly identified roles in other conditions. (Tavera, 2007)

**Figure 3.3 Mechanism of Vitamin D Activity in Cell**

**Cited from Parathyroid Hormone in nephropal .com**

Although most 25-hydroxylase activity is located in the liver, the enzyme is also found in substantially lower quantities in the intestine, adrenal gland, lung, kidney and bone. In addition, although circulating levels of 1,25(OH)2 D are the result of renal 1α–hydroxylation, calcitriol is also synthesized “locally” by 1α-hydroxylases in colon, breast,

prostate, lung, activated macrophages and parathyroid (Holick, 2004). Calcitriol produced by the kidney functions as an endocrine hormone, regulating serum calcium concentrations, while locally produced calcitriol acts in an autocrine and paracrine manner, specifically with respect to cell differentiation (Lips, 2006).

## Physiologic Role of Calcitriol

### Calcium homeostasis and bone metabolism

The endocrine hormone role of vitamin D on calcium homeostasis is the first discovered function of vitamin D. Plasma calcium concentrations are usually tightly maintained between 8.5-10.5 mg/dL. When serum calcium levels drop below 8.5 mg/dL, the parathyroid gland responds by releasing parathyroid hormone (PTH), which triggers a number of processes that help restore serum calcium level back to normal. PTH results in the activation of the renal 1α-hydroxylase, subsequently increasing the production of calcitriol from 25-hydroxyvitamin D. Calcitriol then increases absorption of calcium from small intestine, increases bone resorption by activation of osteoclasts, and increases renal calcium re-absorption (Holick, 2004).

Calcitriol works in several ways to increase serum calcium concentrations to normal levels. Calcitriol:

— binds to VDRs on intestinal enterocytes. It increases expression of epithelial calcium channels and increases synthesis of calbindin and other proteins, which will facilitate calcium transport across the enterocyte into the circulation (Holick, 2004).

— similarly binds to VDRs on renal cells to reduce renal calcium excretion.

— works with PTH to cause an increase in bone resorption. Calcitriol binds to VDRs expressed in osteoblasts, causing them to release factors that lead to the differentiation of precursor cells into mature osteoclasts. As osteoclasts break down

bone by releasing hydrochloric acid and collagenase, calcium is freed from hydroxyapatite, the bone mineral complex, and released into the circulation (Holick, 2004).

The metabolic responses to prolonged vitamin D deficiency result in increased bone resorption and skeletal weakening.

**Nonskeletal function**

Recent research has identified numerous tissues, including immune cells, colon, placenta, breast, prostate, pancreas and the skin, that express the 1α-hydroxylase enzyme and are therefore capable of producing calcitriol (Martini & Wood, 2006). The VDR has also been identified in a number of cells and organs, such as activated T cells and B cells, β-islet cells, and cells in the prostate, breast, colon, and other organs (Holick, 2004a). These new discoveries shed light on the nonskeletal functions of vitamin D, in which it acts as an autocrine and paracrine hormone.

**Cell proliferation and differentiation in relation to cancer**

Since the 1980s, evidence from various cell lines has demonstrated that vitamin D may protect against various cancers, including colon, prostate and breast (Holick, 2008c;Holick 2006). Many epidemiologic studies have also shown a strong inverse relation between exposure to sunlight and the incidence of certain types of cancer. Tanaka et al. (1982) found that calcitriol at concentrations of 0.12 nmol/L and above inhibited the proliferation of human leukemic cells by 50% and induced their differentiation into mature granulocytes. Since then, the anti-proliferative effect of calcitriol has been confirmed in many other cell types (Trump, 2004.). Investigators

found that calcitriol works through both autocrine and pararcrine mechanisms to inhibit proliferation and induce differentiation of normal and some types of cancerous cells (Holick, 2004a).

Based on these findings, researchers hypothesized that calcitriol may have anti-tumorigenic properties and that calcitriol and its analogues could be useful in treatment of cancer. However, tumors develop several different ways to prevent antiproliferative activity of calcitriol. For example, some human prostate cancer cells can up- regulate the 24-hydroxylase, which degrades of calcitriol, thereby inhibiting the effects of calcitriol on proliferation and differentiation. (Chen and Holick, 2003)

**Heart disease**

In 1997, Rostand (1997) first reported that living at ahigh latitude correlated with hypertension. Hypertensive patients exposed to UVB had lower systolic and diastolic blood pressure, which was also accompanied by a 180% increase in 25 (OH) D levels. The mechanism supporting this observation was provided by Li et al (2002). They found calcitriol may suppress renin in a mouse model. Renin is an enzyme that triggers a series of events that result in the production of angiotensin II, which increases blood pressure. This may help to explain the antihypertensive function of vitamin D.

Other studies reported that vitamin D deficiency increases the risk of developing type 2 diabetes (Carbone, 2007), which can exacerbate lipoprotein disorders and increase the risk of cardiovascular diseases. Moreover, Wang et al. (2008) reported that vitamin D deficiency was associated with a 50% increase in the risk of dying from myocardial infarction. Therefore, it is possible that vitamin D deficiency contributes to CVD through its association with risk factors, such as diabetes and hypertension.

Direct effects of vitamin D on the cardiovascular system may also be involved. The VDR is expressed in cardiomyocytes, vascular smooth muscle cells and endothelial cells, and vitamin D has been shown to affect inflammation, cell proliferation and differentiation, which also contribute to heart disease, although the precise relationship has not been described.

**Diabetes**

The presence of 1α-hydroxylase in the pancreas and the expression of the VDR in pancreatic β-cells suggests that calcitriol may be involved in the insulin response. In a study conducted in 51 regions worldwide, researchers showed a relationship between vitamin D deficiency and increased risk of developing type 1diabetes (Mohr, 2008). Furthermore, higher vitamin D intake in early childhood may be associated with decreased risk of developing type 1 diabetes mellitus (Holick, 2008b). Ayesha et al (2001) also found vitamin D deficiency reduces insulin secretion and turnover in rats.

**Autoimmune diseases**

Resting T cells and B cells do not express the VDR, but VDR is expressed in activated B cell, T cells and macrophages, which suggests that vitamin D is involved in immune system regulation on some level (Van and Mathieu, 2005)

Multiple sclerosis, Crohn's disease, inflammatory bowel disease, and ulcerative colitis are all autoimmune diseases. Epidemiologic evidence suggests that these conditions may be related to vitamin D deficiency (Holick, 2004; Sentongo, 2001)

**Infection and inflammation**

The immune system is responsible for protecting the host from infection. There are two main divisions of the immune system: innate immunity, which provides an immediate, but non-specific response to foreign substances; and adaptive immunity, which is activated after the innate response is initiated. Vitamin D is involved primarily with innate immunity, which includes the production of antimicrobial peptides, such as B-defensins and cathelicidins, which are capable of killing viruses, bacteria and fungi. These two particular peptides are produced by epithelial surfaces and within circulating leukocytes (Adams, 2002). Human cathelicidin antimicrobial protein hCAP18, which is the sole cathelicidin protein in humans, is encoded by CAMP. Liu et al (2006) found that hCAP18 is a multifunctional protein with receptor –mediated vitamin D-dependent effects. (Gombart, 2009)

The expression of VDR and the 1-α- hydroxylase gene is up-regulated when receptors in human macrophages are activated, which increases the amount of 1, 25(OH) D in the cell. Thus, calcitriol is able to interact with the promoter region on the cathelicidin gene, enhancing hCAP-18 production and subsequent pathogen destruction. This mechanism has also been demonstrated in myeloid cells (Gombart, 2005), bronchial epithelial cells (Yim, 2007) and keratinocytes ( Weber ,2005). Weber found that 25(OH) D could also induce intracellular hCAP-18 through autocrine induction of the 1-α- hydroxylase enzyme.

Liu et al ( 2006) observed that cathelicidin messenger RNA induction is lower in African-American individuals, who are known to have increased susceptibility to tuberculosis (Matsuoka, 1991) and are more likely to have low 25-hydroxyvitamin D status (Stead, 1990). Differences in the ability of human populations to produce vitamin

D may contribute to susceptibility to microbial infection and inflammatory processes. The relationship between vitamin D status and immune function may help to explain the reported association between vitamin D status and asthma.

Vitamin D also has functions in the adaptive immune system. VDR was found to be expressed in activated T and B lymphocytes. (Bhalla, 1983) 1, 25(OH) D suppresses T helper cell proliferation and modulates their cytokine production such as IL-2 , IL10.( Lemire, 1985). It was also found that 1, 25(OH) D has the ability to inhibit $T_H$ 1 cytokines production (Lemire, 1995) and enhance TH 2 cytokines (Boonstra, 2001), but it suggested that high levels of vitamin D would be associated with asthma, the opposite function in compare with vitamin function in the innate immune system

## Intake Recommendations

The Food and Nutrition Board (FNB) established Adequate Intake levels (AI) for vitamin D that represent a daily intake that is thought to be sufficient to maintain bone health and normal calcium metabolism in healthy persons. The AI for Vitamin D for different ages, which was established in 1997, is shown in Table 3-2.

**Table3-2: Adequate Intakes (AIs) for Vitamin D**

| Age | Children | Men | Women | Pregnancy | Lactation |
|---|---|---|---|---|---|
| Birth to 13 years | 5 mcg (200 IU) | | | | |
| 14-18 years | | 5 mcg (200 IU) | 5 mcg (200 IU) | 5 mcg (200 IU) | 5 mcg (200 IU) |
| 19-50 years | | 5 mcg (200 IU) | 5 mcg (200 IU) | 5 mcg (200 IU) | 5 mcg (200 IU) |
| 51-70 years | | 10 mcg (400 IU) | 10 mcg (400 IU) | | |
| 71+ years | | 15 mcg (600 IU) | 15 mcg (600 IU) | | |

In October of 2008, the American Academy of Pediatrics released new guidelines concerning vitamin D for infants, children and adolescents that doubled the recommended minimum intake of vitamin D from 200 international units (IU) to 400 IU per day because medical conditions attributable to low vitamin D levels appear to be on the rise in the U.S. The FNB also established an expert committee in 2008 to review the DRIs for vitamin D. In recent years, many vitamin D experts have indicated that current AI for vitamin D intake is too low, suggesting that the amount should be increased to 1000 IU/day to raise serum vitamin D to optimal levels (Tangpricha et al.2003) The FNB expects to issue its report, updating as appropriate the DRIs for vitamin D in 2010.

## Assessment of Vitamin D Status

Vitamin D status in the body is assessed by the measuring circulating levels of $25(OH)D_3$ . The traditional deficiency values were defined by The Institute of Medicine in 1997s as less than 27.5 nmol/L in children and less than 37.5 nmol/L in adults. However, many scientists have suggested that the criteria used to define deficiency status should be revised since these levels were set based on adequate amounts to prevent rickets in children and osteomalacia in adults, and not the nonskeletal functions of vitamin D, such as its role in cancer, autoimmunity and heart disease (Heaney, 2003). Because vitamin D plays a central role in calcium homeostasis and skeletal health, most researchers agreed that optimal range of circulating $25(OH)D_3$ should be the range that reduces PTH levels to a minimum and maximizes calcium absorption (Dawson-Hughes et al. 2005). Several studies have shown that circulating $25(OH)D_3$ levels of approximately 75 nmol/L are sufficient to optimize vitamin D status. (Chapuy, 1997; Holick, 2005,Heaney, 2003).

## Vitamin D deficiency

Because humans can synthesize vitamin D from sun exposure, the potential for widespread deficiency was previously thought to be quite small. However, recent studies have begun to reveal the unrecognized high prevalence of vitamin D deficiency in many populations worldwide (Ginde, 2009;Gordon, 2004; Looker, 2002; Kinyamu, 1997; Chapuy, 1997). Vitamin D inadequacy and deficiency are reported in a wide variety of apparently healthy populations, including children, adolescents, young adults, and middle-aged and elderly adults. Dark-skinned and older persons are at the highest risk of deficiency because pigment in dark skin reduces the amount of UVB that reaches 7-dehydrocholesterol and the level of 7-dehydrocholesterol in the skin declines with age (Holick, 2004a).

The reasons for widespread vitamin D deficiency are not completely understood, but researchers believe that changes in lifestyle play an important role. Most people do not spend sufficient time in the sun without wearing sunscreen to produce enough vitamin D. In addition, as discussed previously, few foods are naturally rich in vitamin D and only a handful of foods are fortified, so dietary intake of the vitamin is frequently inadequate.

Prolonged vitamin D deficiency decreases calcium absorption, leading to bone abnormalities, such as rickets in children and osteomalacia in adults. (Lips, 2001). Rickets is characterized by softening and weakening of bones, leading to bowed legs, knocked knees and rib bone malformation. The prevalence of rickets was extremely high in the nineteenth century due to industrialization and child labor practices that frequently limited sun exposure. Although rickets is rare in developed countries, numerous cases

have been reported in exclusively breastfed infants, particularly African-Americans, and the condition continues to be a problem in developing countries. Osteomalacia is a similar condition that occurs in adults and contributes to fractures. (Thacher et al., 2006)

# CHAPTER 4

# VITAMIN D AND ASTHMA

The possible relationship between asthma and vitamin D deficiency was first put forward by Litonjua and Weiss in 2007. It is a new area of research, and few studies have examined the potential relationship, but results from these studies provide evidence that vitamin D status may influence the risk of developing asthma (Camargo, 2007; Devereux, 2007; Erkkola 2009; Camargo, 2006; Hypponen, 2004; Gale, 2007).

## Innate Immunity, Infection and Vitamin D

Human infections are caused by viruses, bacteria, and fungi. When potential pathogens enter in the body, the innate immune system is designed to recognize non-specific pathogen associated molecular patterns (PAMP's). The vast majority of microorganisms are destroyed within minutes or hours by innate defenses. The adaptive immune response comes into play only with specific signaling of the innate immune system. Innate immunity, therefore, is the first line of defense against infection (Fleer, 2007). Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system. They are single membrane-spanning, non-catalytic receptors that recognize structurally conserved molecules derived from microbes. Once these microbes have breached physical barriers, such as the skin, lung epithelial surface or intestinal mucosa, they are recognized by TLRs, which activates immune cell responses (Hewison, 2008). Human cathelicidin antimicrobial protein hCAP18 is one of the TLR

proteins in the innate immune system, and it is expressed in epithelial cells of the human lung (Bals, 1998). Immunohistochemistry has demonstrated that, in addition to bronchial epithelial cells, hCAP18 is located in alveolar macrophages and bronchial glands (Agerberth, 1999). This important antimicrobial peptide is mediated by vitamin D.

Liu et al (2006) found that hCAP18 is has receptor–mediated vitamin D-dependent effects. The expression of the VDR and 1-α-hydroxylase genes is up-regulated when receptors in human macrophages are activated, which increases the amount of 1, 25(OH) D in the cell. Thus, locally produced calcitriol is able to interact with the promoter region on the cathelicidin gene, enhancing hCAP-18 production and subsequent ability to recognize pathogens. This mechanism has also been demonstrated in bronchial epithelial cells (Yim, 2007). Pathogens would be largely decreased through the action of hCAP-18.

## Studies Examining Vitamin D and Asthma

Litonjua and Weiss first suggested that vitamin D deficiency in pregnant women and their offspring could at least partly explain the current rise in incident asthma. (Moorman, 2007) Although subsequent epidemiologic studies provided additional support for an association between vitamin D status and asthma, study results have been inconsistent, with some evidence suggesting no association and others even suggesting that vitamin D might be detrimental.

A large cross-sectional study from the Third National Health and Nutrition Examination Survey in U.S. reported that vitamin D intakes and serum levels of 25 (OH) vitamin D were associated with lung function in adults (Black, 2005). Similar findings have been reported in Australian adolescents (Burns, 2006). In addition, an association

between low maternal intakes of vitamin D during pregnancy and early childhood wheezing has been reported in studies in the United States (Camargo, 2007) and Scotland (Devereux, 2007). Both studies had a large sample size (over 1000 pregnant participants) and showed that the risk of wheeze was more than 50% lower in offspring whose mother had higher vitamin D intake compared to those whose mothers had lower intake. However, although the Scottish study (Devereux, 2007) reported an association between low vitamin D intake and increased risk of respiratory infections and childhood wheezing, no association was observed between vitamin D intake and asthma diagnosed at the age 5. This finding is in contrast to the results of another recent birth cohort study, conducted in Finland among 1669 mother and child pairs (Erkkola 2009), which found that higher maternal vitamin D intake during pregnancy was associated with a reduced risk of asthma in 5-year-old children.

An important weakness in these studies is that they all relied solely on food frequency assessment of vitamin D intake as their measure of vitamin D status; none of the studies measured serum 25 (OH) D level, which is a much more reliable measure of vitamin D status. Dietary vitamin D intake is actually poorly correlated with serum levels of 25-OH D, which is influenced by many non-dietary factors, such as skin pigmentation, latitude and the extent of sun exposure. Although these studies raise the possibility that dietary vitamin D intake may influence the risk of developing asthma, it is also possible that the observed associations may be confounded by other components in vitamin D-rich foods, such as omega-3 fatty acids, that may impact airway reactivity (Reisman et al., 2006). In addition, persons with asthma may adopt certain lifestyle characteristics, such as avoiding outdoor exercise and milk intake (Camargo, 2007), that could influence the occurrence of asthma or estimates of vitamin D intake. One study that did measure 25(OH) vitamin D also reported inconsistent findings. In a birth cohort study, conducted

among 922 infants in New Zealand (Camargo, 2008), low cord blood levels of 25(OH) D levels were associated with increased respiratory infections and childhood wheezing but not with asthma diagnosed at age 5.

In addition to studies suggesting that vitamin D may protect against asthma or have no effect, a few studies have actually reported that vitamin D might increase asthma risk. Hypponen et al. (2004) reported that regular vitamin D supplementation (≥ 2000 IU/d) in the first year of life increased the risk of developing asthma by age 31 years (Hypponen, 2004). However, this study did not assess maternal vitamin D intake nor did it measure serum 25(OH) vitamin D levels in the subjects and their mothers. Therefore, we do not know whether the vitamin D supplementation increased the asthma risk or if the infant's asthma risk was influenced by maternal vitamin D status during pregnancy. It is also possible that the long follow-up period contributed to recall bias, which could affect estimates of the association. Another prospective study, conducted among 466 pregnant women in the United Kingdom, also reported that high 25(OH)-vitamin D concentration in the peripheral circulation during late pregnancy was associated with an increased risk of eczema at 9 months and asthma at age 9 years (Gale, 2007). A weakness of this study is that it had a large loss to follow up, with only 30% of the participants retained at the end, and it is possible that the vitamin D status or occurrence of asthma differed between those who were lost to follow up and those who completed the study. These studies suggest that maternal supplemental vitamin D and high serum vitamin D concentrations may somehow increase the risk of developing asthma in their offspring (Wjst, 2006).

In addition to the development of asthma, some researchers have focused on examining the relationship between vitamin D and asthma severity in persons already

diagnosed with asthma. Two studies involving a childhood asthma management program in the United States (Ciaccio,2010; Brehm, 2010) and one cross-sectional study in Costa Rica ( Brehm, 2009) all suggested that vitamin D insufficiency [serum 25(OH) D <75 nmol/L] was common in children with severe asthma. One limitation of these studies is that none of them had data on respiratory tract infections. It is possible that vitamin D may work indirectly by helping to prevent respiratory tract infections rather than by modifying airway inflammation. However, Xystrakis et al (2006) reported in that vitamin D may enhance glucocorticoid responsiveness by increasing IL-10 production, which suggests that vitamin D may help to ameliorate the inflammatory symptoms associated with asthma. The inconsistency of the findings from these studies underscores the need for further research in this area to clarify the association between vitamin D status and asthma.

# CHAPTER 5

# PURPOSE OF THE STUDY

Asthma is a common condition throughout the world, contributing to both morbidity and healthcare costs. Vitamin D deficiency is also a world health problem, and recent evidence suggests that the two conditions may be related, although the data supporting an association are inconsistent and contradictory.

The main objective of this research project is to assess the relationship between measures of vitamin D status and self-reported wheeze and asthma in a cohort of young, healthy women who participated in the UMass Vitamin D Status Study.

## Hypotheses and Specific Aims

### Hypotheses

1. Higher serum 25(OH)D3 concentration will be associated with reduced risk of wheeze and asthma.
2. Higher dietary intake of vitamin D will be associated with reduced risk of wheeze and asthma.

**Specific aims**

1. Determine the prevalence of suboptimal vitamin D status and vitamin Deficiency among subjects by measuring serum concentrations of 25 (OH) vitamins D.

2. Assess the relationship between dietary intake of vitamin D and other nutrients and self-reported wheeze and asthma.

3. Assess the relationship between serum concentrations of 25(OH) vitamin D and self-reported wheeze and asthma.

4. Identify the predictors of serum 25 (OH) D in this specific sample.

# CHAPTER 6

# MATERIALS AND METHODS

## Study Subjects

The current analyses use data obtained from the University of Massachusetts Vitamin D Status Study, a cross-sectional study conducted by researchers at the University of Massachusetts. The current study includes 186 women between the ages of 18 and 30, mostly from UMass Amherst campus and surrounding community, who were voluntarily enrolled in the study between 2006 and 2008. Amherst is located in Western Massachusetts (latitude, 42.37N and longitude, -72.53W ). Women were excluded from the main study if they were pregnant and/or had any serious chronic diseases, such as heart disease or diabetes, or were currently taking any of the following medications: corticosteroids, anabolic steroids, anticonvulsants, propranolol and cimetidine.

## Data Collection

### Collection of dietary and demographic data

Each participant completed a single 2-hour clinic visit, which was scheduled during the late lacteal phase of her menstrual cycle, three to five days before the expected start of the next menstrual period. At that visit, women completed a previously validated Harvard Food Frequency Questionnaire (Willett, 1985) and a questionnaire designed specifically for this study to elicit demographic and health-related data.

The FFQ asked about common food sources of vitamin D (fortified milk and fatty fish) as well as foods that may or may not contain the vitamin, depending on the manufacturer's fortification practices (yogurt, cheese, breakfast cereals, fruit juices). The Harvard FFQ was adapted for this study to inquire about food intake in the previous two months.

**Measurements**

Height, weight and waist circumference were measured during the clinical visit. Height was measured to the nearest 0.5 centimeter; weight in light clothing was measured to the nearest 0.1 kilogram. Waist circumference was measured to the nearest 0.5 inch. Body mass index (BMI) was calculated as weight (kg)/[height (m)]$^2$. A dual x-ray absorptiometry (DXA) scan was performed by the same trained technician to determine body composition, including percent body fat. Fasting venous blood samples were obtained by a trained phlebotomist. Blood samples were kept on ice for up to two hours prior to centrifugation and separation into serum and plasma fractions. Serum and plasma were stored at -80º C until assayed. Serum concentration of 25(OH) vitamin D was measured in the laboratory of Dr. Alayne Ronnenberg using a commercially available radioimmunoassay kit (Diasorin, MN). The coefficient of variation for this assay in the Ronnenberg lab varied from 8 to 13%. Final determination of 25(OH) vitamin D concentrations were calculated using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California, USA).

**Respiratory symptom data collection**

As part of the health questionnaire, subjects responded to a battery of questions designed to elicit information on respiratory symptoms, including wheeze and the diagnosis of asthma. These questions were derived from previously validated

international study of asthma and allergies in childhood study questionnaire. The questionnaire also ascertained whether the participant had eczema, a condition often associated with asthma.

## Statistical Analysis

The statistical analysis will be carried out using SAS version 9.2. Descriptive data are summarized using mean ± standard deviations, median, range and percentages. Logarithmic transformation was used to improve normality for variables that were not normally distributed. Statistical significance is set at $p \leq 0.05$.

### Main exposure and outcome variables

The main outcome measures were self-reported wheeze and self-reported asthma, both of which were modeled as dichotomous (yes/no) variables. A secondary outcome measure was serum concentration of 25(OH) vitamin D, which was modeled as a continuous variable, a categorical variable (deficient, suboptimal, optimal), and a dichotomous variable (insufficient, sufficient), depending on the specific analysis. The main exposure variables were indicators of vitamin D status—both energy-adjusted dietary intake of vitamin D, modeled as both a continuous and a dichotomous (below AI/at or above AI) variable, and serum 25(OH) vitamin D concentration, also modeled as both a continuous variable and a categorical (deficient, suboptimal, optimal) variable.

### Covariates

The main covariates of interest include BMI, percent body fat, age, and smoking history. Other covariates considered for inclusion in final models include sun exposure, season of blood draw, oral contraceptive (OC) use, activity level, and numerous dietary factors, including intake of vitamins C and E and omega-3 fatty acids.

**Statistical tests**

Chi-Square tests for equivalence of proportions and t-tests for equality of means were used to assess the relationship between covariates and asthma/wheeze and the relationship between covariates and measures of vitamin D status (dietary intake and serum concentration of 25(OH) vitamin D). To achieve the second and third study aims, which are to assess the relationship between dietary intake of vitamin D, serum 25(OH) D and other nutrients and self-reported wheeze and asthma, simple logistic regression was used first to calculate a crude prevalence odds ratio (POR) and 95% confidence interval (CI) between dietary intake of vitamin D and other nutrients and asthma and wheeze. For each univariable logistic model, variables with a p value of ≤ 0.25 were entered into multiple logistic regression models. Final models describing each of the relationships include covariates that are significant at the p≤ 0.10 level as well as other variables, such as BMI and smoking status, which may be considered important components of the model. The results of multiple logistic regressions are expressed as odds ratios (OR) with 95% CI.

Linear regression was used to identify the predictors of serum 25(OH)D in our sample. Simple linear regression was used to identify potential predictors; variables that were associated with vitamin D at the level of p ≤ 0.25 were included in multivariable linear regression models. The criteria for variable inclusion in the final regression model was similar to that used for multiple logistic regression, described above.

# CHAPTER 7

# RESULTS

There were 186 women enrolled in the study, most of whom (85.5%) were white (Table 7.1). The mean age was 21.5 (SD=3.2) years and the mean BMI was 23 kg/$m^2$. Mean dietary intakes of vitamin D and energy were 375 IU and 2187 kcal, respectively.

Characteristics of study participants based on optimal ( ≥75nmol/L n=84) versus suboptimal (< 75nmol/L n=102) serum levels of 25-OH vitamin D are also included in Table 7.1. Although the mean (± SD) levels of most covariates did not differ by vitamin D status, dietary vitamin D intake was significantly greater (p =0.04) in the group with optimal vitamin D status than in the group with suboptimal serum levels of 25-OH vitamin D. A total of 82 participants reported having experienced wheeze or asthma symptoms in the past 12 month. Of these, 37(45%) had suboptimal serum 25(OH) D and 45(55%) had optimal vitamin D status. The proportion of women with suboptimal vitamin D status differed significantly according to sun exposure (p<0.02) and race (p< 0.01).

Characteristics of the study sample based on wheeze/asthma symptoms are shown in Table 7.2. Older, more active women and those with lower serum 25(OH) D levels reported fewer wheeze/asthma symptoms. In addition, the group with asthma/wheeze symptoms had significantly higher serum 25(OH) D status (p=0.02) than women without symptoms. The proportion of women who had low dietary vitamin D

intake, short time (≤ 2 hours) spent outside in minimal clothing and high hs-CRP level were significantly higher in women with symptoms than in those without.

**Table 7.1 Distribution of participant characteristics by serum 25(OH)D**

| Variable | Total | Serum vitamin D status suboptimal | Serum vitamin D status optimal | p-value[1] |
|---|---|---|---|---|
| | n=186 | n=102 | n=84 | |
| | Mean (SD) | Mean (SD) | Mean (SD) | |
| **Age( years)** | 21.5(3.2) | 21.8(3.4) | 21.3(2.9) | 0.32 |
| **BMI（kg/m$^2$）** | 23.0(3.2) | 23.1(3.3) | 23.0(2.9) | 0.76 |
| **Body Fat (%)** | 31.9(7.9) | 32.4(8.1) n=97 | 31.3(7,7) n=80 | 0.35 |
| **Physical Activity (MET$^2$/week)** | 9.0(3.6) | 9.4(3.7) | 8.6(3.3) | 0.21 |
| **Dietary intake** | | | | |
| **Vitamin D ( IU)** | 375(269) | 338(234) | 421(303) | **0.04** |
| **Vitamin C (mg)** | 208(180) | 209(157) | 206(205) | 0.92 |
| **Vitamin E (mg)** | 18.4(29.9) | 18.0(34.1) | 18.9(24.1) | 0.83 |
| **Omega-3 (g)** | 0.3(0.3) | 0.3(0.3) | 0.3(0.3) | 0.72 |
| **Calcium (diet; mg)** | 1018(475) | 1029(483) | 1005(466) | 0.74 |
| **Total Energy (Cal)** | 2187(785) | 2187(808) | 2187(761) | 1 |
| | n (%) | n (%) | n (%) | |
| **Vitamin Intake by DRI[3]** | | | | |
| **Vitamin D (< 200 IU)** | 54 (29.0) | 34 (63) | 20 (37) | 0.15 |
| **Vitamin C (< 60 mg)** | 8(4.3) n=168 | 7(88) | 1(12) | 0.08 |
| **Vitamin E (< 15 mg)** | 110(59.2) | 68(62) | 42(38) | **0.02** |
| **BMI** | | | | 0.79 |
| **≤25 (kg/m$^2$)** | 140(75.3) | 76(54) | 64(46) | |
| **>25 (kg/m$^2$)** | 46(24.7) | 26(57) | 20(43) | |
| **hs CRP ≥3 mg/L** | 49(26.3) | 20(41) | 29(59) | **0.02** |
| **Respiratory symptom** | | | | **0.02** |
| **Wheeze or asthma** | 82(44.1) | 37(45) | 45(55) | |
| **No symptom** | 104(55.9) | 65(63) | 39(37 ) | |

**Table 7.1, Continued**

| Variable | Total | Serum vitamin D status suboptimal | Serum vitamin D status optimal | p- value |
|---|---|---|---|---|
| | n=186 | n=102 | n=84 | |
| | n(%) | n (%) | n (%) | |
| **Race** | | | | **0.01** |
| **White** | 159(85.5) | 81(51) | 78(49) | |
| **Non-white** | 27(14.5) | 21(78) | 6(22) | |
| **Education** | | | | 0.11 |
| **Some college** | 150(80.7) | 78(52) | 72(48) | |
| **College graduate** | 36(19.3) | 24(67) | 12(33) | |
| **Smoking Status** | | | | 0.91 |
| **Ever/current smoker** | 26(14.0) | 14(54) | 12(46) | |
| **Non- smoker** | 160(86.0) | 88(55) | 72(45) | |
| **Current oral contraceptives use** | 73(39.3) | 26(36) | 47(64) | **<0.0001** |
| **Hours outdoors in past week** | | | | 0.16 |
| **≤ 2 hours** | 156(83.9) | 89(57) | 67(43) | |
| **≥ 3 hours** | 30(16.1) | 13(43) | 17(57) | |
| **No sunscreen use** | 76(40.9) | 38(50) | 38(50) | 0.27 |
| **Month of blood draw** | | | | **0.02** |
| **High sun exposure month[3] (4,5,9,10)** | 99 | 46(46) | 54(54) | |
| **Low sun exposure month(1,2,3,11,12)** | 84 | 54(64) | 30(36) | |

1. Statistical significance was assessed using t-test for continuous variables and chi-squarer analysis for categorical variables

2. MET: Metabolic Equivalent, a unit used to estimate the amount of oxygen used by the body during physical activity.

3. DRI: Dietary Recommended Intakes (AI and RDA) for women in this age range. The AI for vitamin D =200IU; the RDA for vitamin C=60mg, vitamin E=15mg

4. No participants was draw the blood in summer break( June, July, August)

**Table 7. 2 Distribution of participant characteristics by wheeze/asthma symptoms**

| Variable | Total | Wheeze/asthma symptoms | No symptoms | p- value |
|---|---|---|---|---|
| | n=186 | n=82 | n=104 | |
| | Mean (SD) | Mean (SD) | Mean (SD) | |
| **Age( years)** | 21.5(3.2) | 20.9( 2.2) | 22.0(3.8) | **0.02** |
| **BMI（kg/m$^2$）** | 23.0(3.2) | 23.4(2.9) | 22.8(3.3) | 0.19 |
| **Body Fat (%)** | 31.9(7.9) | 33.1(6.9) (n=78) | 31.0(8.5) (n=99) | 0.07 |
| **Physical Activity (MET[1]/week)** | 9.0(3.6) | 8.4(2.5) | 9.5(4.3) | **0.02** |
| **Serum 25(OH)D** | 79.3(31.8) | 85.7(33.2) | 74.0(29.8)n=100 | **0.01** |
| **Dietary intake** | | | | |
| **Vitamin D ( IU)** | 375(269) | 349.3(277.1) | 396.0(262.7) | 0.24 |
| **Vitamin C (mg)** | 208(180) | 224.2(223.6) | 194.9(135.4) | 0.30 |
| **Vitamin E (mg)** | 18.4(29.9) | 17.6(24.5) | 19.1(33.7) | 0.74 |
| **Omega-3 (g)** | 0.30(0.30) | 0.30(0.30) | 0.30(0.30) | 0.44 |
| **Calcium, no supplement (mg)** | 1018(475) | 1029(533) | 1010(425) | 0.79 |
| **Total Energy (kcal)** | 2187(785) | 2279(866) | 2115(711) | 0.15 |
| | n (%) | n (%) | n (%) | |
| **Dietary frequency by DRI[2]** | | | | |
| **Vitamin D suboptimal** | 54(29.0) | 31(57) | 23(43) | **0.02** |
| **Vitamin C suboptimal** | 8(4.3) n=168 | 5(63) | 3(37) | 0.28 |
| **Vitamin E suboptimal** | 110(59.2) | 48(44) | 62(56) | 0.88 |
| **BMI** | | | | 0.44 |
| **≤25 (kg/m$^2$)** | 140(75.3) | 64(46) | 76(54) | |
| **>25 (kg/m$^2$)** | 46(24.7) | 18(39) | 28(61) | |
| **hs CRP>3 mg/L** | 49(26.3) | 27(55) | 22(45) | 0.07 |
| **Vitamin D status 25(OH)D** | | | | **0.02** |
| **<75nmol/L** | 102(54.9) | 37(36) | 65(64) | |
| **≥75nmol/L** | 84(45.2) | 45(54) | 39(46) | |
| **Race** | | | | 0.96 |
| **White** | 159(85.5) | 70(44) | 89(56) | |
| **Non-white** | 21(14.5) | 12(44) | 15(56) | |

**Table 7.2 continued**

| Variable | Total | Wheeze/asthma symptoms | No symptoms | p- value |
|---|---|---|---|---|
| | n=186 | n=82 | n=104 | |
| | n (%) | n (%) | n (%) | |
| **Time outdoors in past week** | | | | 0.007 |
| **≤ 2 hours** | 156(83.9) | 62(40) | 94(60) | |
| **≥ 3 hours** | 30(16.1) | 20(67) | 10(33) | |
| **No sunscreen use** | 76(40.9) | 31(41) | 45(59) | 0.45 |
| **Education** | | | | 0.15 |
| **Some college** | 150(80.7) | 70(47) | 80(53) | |
| **College graduate** | 36(19.3) | 12(33) | 24(67) | |
| **Smoking Status** | | | | 0.51 |
| **Ever/current smoker** | 26(14.0) | 13(50) | 13(50) | |
| **Non- smoker** | 160(86.0) | 69(43) | 91(57) | |
| **Current oral contraceptives use** | 73(39.3) | 36(49) | 37(51) | 0.25 |

1. MET: Metabolic Equivalent, a unit used to estimate the amount of oxygen used by the body during physical activity.

2. DRI: Dietary Reference Intake (includes adequate intake, AI, and recommended dietary allowance, RDA. The AI for vitamin D =200 IU; the RDAs for vitamin C=60mg and vitamin E=15mg

Univariable logistic regression was used to assess the relationship between wheeze/ asthma symptoms and each exposure variable (Table 7.3). Physical activity level, in Metabolic Equivalents (METS; used to estimate the amount of oxygen consumed during physical activity) and total energy intake were divided into quartiles. Suboptimal serum 25(OH)D status, short time (≤ 2 hours) daily spent outside and high physical activity (the highest quartiles of physical activity level) were significantly associated with reduced risk of wheeze/asthma symptoms ($p<0.05$), whereas low dietary vitamin D and high hs-CRP were associated with increased risk of symptoms ($p =0.02$ and 0.07, respectively).

Variables with $p \leq 0.25$ in univariable models as well as BMI, energy intake and smoking status, which were considered important components of the model for physiologic reasons, were entered into a multivariable logistic regression model. (Model 1, Table 7.4). After adjusting for other covariates, all previously significant variables

except physical activity remained significantly associated with wheeze/asthma (p <0.1; Model 2) and were entered into Model 3. Controlling for energy, BMI, smoking and age (Model 4) did not substantially alter OR estimates or the level of statistical significance.

**Table 7.3 Univariable Logistic Regression by Wheeze/Asthma Symptoms**

| Variable | Unadjusted OR (95%) | p- value |
|---|---|---|
| Education | | **0.15** |
| Some college | 1.75(0.82,3.76) | |
| College graduate | 1 | |
| BMI（$kg/m^2$） | | 0.44 |
| ≤25 ($kg/m^2$) | 1 | |
| >25 ($kg/m^2$) | 0.76(0.39,1.51) | |
| Body Fat (%) | | |
| ≤32 | 1 | 0.88 |
| >32 | 1.05(0.59,1.87) | |
| Physical Activity ($MET^1$/week)[3] | | |
| Quartile 1 | 1 | 1 |
| Quartile 2 | 1.14(0.51,2.59) | 0.65 |
| Quartile 3 | 1.55(0.68,3.52) | **0.10** |
| Quartile 4 | 0.61(0.26,1.42) | **0.05** |
| Serum 25(OH) D | | |
| <75 nmol/l | 0.49(0.27,0.89) | **0.02** |
| ≥75 nmol/L | 1 | 1 |
| Dietary vitamin D | | |
| <200IU | 2.14(1.13,4.07) | **0.02** |
| ≥200IU | 1 | 1 |
| hs CRP>3 mg/L | 1.83(0.95,3.54) | **0.07** |
| Time outside | | |
| ≤ 2 hours | 0.33(0.15,0.75) | **0.008** |
| ≥ 3 hours | 1 | 1 |
| Current oral contraceptives use | 1.42(0.78,2.56) | 0.24 |
| Energy Intake[3] | | |
| Quartile 1 | 1 | 1 |
| Quartile 2 | 1.38(0.60,3.16) | 0.91 |
| Quartile 3 | 1.20(0.52,2.78) | 0.67 |
| Quartile 4 | 1.94(0.85,4.44) | **0.15** |

1. MET: Metabolic Equivalent, a unit used to estimate the amount of oxygen used by the body during physical activity.
2. RDA: Reference Daily Allowance .The RDA for vitamin D =200IU, vitamin C=60mg, vitamin E=15mg
3. Quartile 1: cut off lowest 25% of data, Quartile 2: cut data set in half, Quartile 3: cut at 75% of data, Quartile 4: cut off highest 25% of data.

**Table 7.4 Multivariable Logistic Regression of wheeze/asthma symptom by selected covariates**

| | Univariable[1] | | Multivariable | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Model 1 | | Model 2 | | Model 3 | | Model 4 | |
| **Variable** | **OR (95%CI)** | **p-value** | **OR (95%CI)** | **p-value** | **OR (95%CI)** | **p-value** | **OR ( 95%CI)** | **p-value** | **OR (95%CI)** | **p-value** |
| **Low serum 25(OH)D (<75nmol/l)** | 0.49 (0.27,0.89) | 0.02 | 0.48 (0.24,0.96) | **0.04** | 0.45 (0.22,0.91) | **0.03** | 0.50 (0.27,0.95) | 0.03 | 0.52 (0.27,0.98) | **0.04** |
| **Low vitamin intake(<200IU)** | 2.14 (1.13,4.07) | 0.02 | 3.35 (1.61,6.96) | **0.001** | 3.63 (1.72,7.63) | **0.0007** | 2.93 (1.46,5.90) | 0.003 | 3.13 (1.54,6.39) | **0.002** |
| **Hs_CRP (>=3mg/l)** | 1.83 (0.95,3.54) | 0.07 | 2.06 (0.94,4.51) | **0.07** | 1.97 (0.89,4.35) | 0.1 | 2.06 (1.00,4.22) | 0.05 | 1.96 (0.94,4.12) | 0.07 |
| **Time outside <=2hours** | 0.33 (0.15,0.75) | 0.008 | 0.28 (0.11,0.68) | **0.005** | 0.30 (0.12,0.74) | **0.009** | 0.27 (0.11,0.66) | 0.004 | 0.29 (0.12,0.72) | **0.007** |
| **High physical activity (Mets >10.13/week)** | 0.50 (0.25,1.01) | 0.05 | 0.50 (0.19,1.34) | 0.17 | 0.89 (0.28,2.89) | 0.85 | | | | |
| **Low education level( some college)** | 1.75 (0.82,3.76) | 0.15 | 1.19 (0.41,3.48) | 0.75 | 2.53 (0.63,10.18) | 0.19 | | | | |
| **Current oral contraceptives user** | 1.42 (0.78,2.56) | 0.24 | 0.98 (0.47,2.05) | 0.96 | 0.92 (0.44,1.94) | 0.83 | | | | |
| **High energy intake ( >2614kcal)** | 1.94 (0.85,4.44) | 0.15 | 1.86 (0.89,3.93) | 0.11 | 1.95 (0.91,4.15) | 0.09 | | | | |
| **High BMI (>25kg/m$^2$)** | 0.76 (0.39,1.51) | 0.44 | 0.68 (0.32,1.47) | 0.32 | 0.62 (0.29,1.35) | 0.23 | | | 0.63 (0.30,1.35) | 0.24 |
| **Ever and current smoker** | 0.76 (0.33,1.74) | 0.51 | 0.67 (0.26,1.72) | 0.4 | 0.57 (0.21,1.52) | 0.26 | | | 0.67 (0.26,1.72) | 0.40 |
| **Age(years)** | 0.90 ( 0.81, 0.99) | 0.03 | | | 0.83 (0.66,1.03) | 0.09 | | | 0.89 (0.80,1.00) | **0.05** |

1. Individual univariable logistic regression models with variable p<0.25 and some important components
2. Model 1: Multivariable logistic regression model with variable p<0.25 and some important components.
3. Model 2: adjust age for Multivariable logistic regression mode 1
4. Model 3: Multivariable logistic regression model with variable p<0.1 in model 2
5. Model 4: adjust age , BMI, smoking for multivariable logistic regression 3

We used univariable linear regression to assess associations between exposure variables and serum 25 (OH)D (Table 7.5). Dietary vitamin D, blood draw in a high sun-exposure month, cold cereal and milk intake and current OC use were significantly positively associated with serum 25 (OH) D, whereas BMI, sunscreen use and <2 hours outside daily were negatively associated with 25(OH) D levels ($p < 0.25$). When these variables were entered in multivariable linear regression models (Table 7.6, Models 2 & 3), four variables were significantly positively associated with vitamin D levels after controlling for energy intake: dietary vitamin D, cold cereal intake, OC use and blood draw in a high sun-exposure month; sunscreen use was negatively associated with 25(OH) D.

**Table 7.5 Univariable linear regression of serum 25(OH) D by main factors**

| | Coefficient | $R^2$ | p-value |
|---|---|---|---|
| **Dietary Vitamin D** | 0.02 | 0.04 | **0.006** |
| **BMI** | -1.03 | 0.01 | **0.17** |
| **Calcium without supplement** | -0.0001 | 0.000003 | 0.98 |
| **Omega 3** | 1.2 | | 0.88 |
| **F205** | -1.05 | 0.000016 | 0.96 |
| **F225** | 74.19 | 0.004 | 0.37 |
| **F226** | 3.58 | 0.0004 | 0.78 |
| **Sunscreen use** | -7.21 | 0.012 | **0.13** |
| **Current oral contraceptive use** | 23.69 | 0.13 | **<.0001** |
| **Time outside (≤2hours)** | -9.08 | 0.01 | **0.15** |
| **Blood draw in high sun exposure month**[1] | 10.67 | 0.17 | **0.03** |
| **Food source** | | | |
| **Skim** | 4.81 | 0.0056 | 0.31 |
| **Milk** | 11.48 | 0.02 | **0.06** |
| **Egg** | 3.79 | 0.003 | 0.46 |
| **Liver** | -6.73 | 0.0014 | 0.61 |
| **Tuna** | 0.02 | 0 | 1.0 |
| **Dkfish** | -1.85 | 0.00085 | 0.7 |
| **Cold cereal intake** | 16.37 | 0.065 | **0.00005** |
| **Orange juice** | 0.84 | 0.0016 | 0.86 |
| **Multivitamins** | 7.7 | 0.015 | **0.10** |

1. High sun exposure months include Apr, May, Sep, Oct. No blood was drawn in June, July or August

**Table7. 6 Multivariable Linear Regression Model of Serum 25(OH)D by Selected Covariables**

| | Univariable | | Multivariable | | | |
|---|---|---|---|---|---|---|
| | | | Model 1[1] | | Model 2[2] | |
| | Coefficient | P-value | Coefficient | P-value | Coefficient | P-value |
| **Dietary vitamin D (IU)** | 0.02 | 0.006 | 0.013 | 0.19 | 0.017 | 0.03 |
| **BMI (kg/m$^2$)** | -1.03 | 0.17 | -1.23 | 0.09 | -1.27 | 0.07 |
| **Sunscreen use** | -7.21 | 0.13 | -9.26 | 0.04 | -9.31 | 0.04 |
| **Oral contraceptive use** | 23.69 | <.0001 | -18.96 | <.0001 | 19.34 | <.0001 |
| **Milk** | 11.48 | 0.06 | 9.43 | 0.1 | 8.69 | 0.13 |
| **Cold cereal** | 16.37 | 0.00005 | 11.51 | 0.02 | 11.26 | 0.02 |
| **Blood draw in high sun exposure month[3]** | 10.67 | 0.03 | -7.8 | 0.1 | 8.86 | 0.05 |
| **Time outside <=2hours** | -9.08 | 0.15 | -4.82 | 0.43 | | |
| **Multivitamin use** | -7.70 | 0.1 | -3.77 | 0.49 | | |
| **Energy** | | 0.88 | 0.0002 | 0.95 | -0.0003 | 0.93 |

1. Model 1 multivariable linear regression model with variable p<0.25 and energy.
2. Model 2 multivariable linear regression model with variable p<0.1 in model 1 and energy.
3. High sun exposure month include Apr, May, Sep, Oct . No blood was drawn in June, July or August.

# CHAPTER 8

# DISSUSION

The prevalence of asthma/wheeze (30.8%) observed in this cross-sectional study is substantially higher than that reported for U.S. white non-Hispanic females (10.6%) or all females (9.6%) in the same age range (NHIS data, 2007). The high prevalence we observed may be due to misclassification of the outcome since wheeze/asthma status was self-reported and was not confirmed by examination of medical records. The relatively high number of wheeze/asthma "cases" in the current study could influence estimates of the association between exposure variables and asthma. Such misclassification, however, would tend to bias our results toward the null.

We found that the risk of wheeze/asthma symptoms was three-times higher among women with low dietary vitamin D intake (<200 IU/day) than in those with higher dietary vitamin D. This observation is consistent with the findings of a birth cohort study in which low maternal vitamin D intake was associated with increased risk of recurrent wheezing in children. (Litonjua, 2007, Camargo, 2007; Devereux, 2007; Erkkola ,2009).

We hypothesized that high serum 25(OH) D would similarly be associated with reduced risk of asthma/wheeze, but we found the opposite effect: after controlling for vitamin D intake and other covariates, the risk of asthma/wheeze was 48% *lower* among women with "suboptimal" serum levels of 25(OH)D compared to women with optimal vitamin D status. Although our findings were unexpected, they lend some support to

previous observations by Gale et al.(2007), which found that high 25(OH) D concentration in the peripheral circulation during late pregnancy was associated with increased risk of eczema at 9 months and asthma at age 9 years. It is unclear whether higher serum levels of 25(OH) are actually detrimental. We speculate that women in our study who had higher serum levels of vitamin D likely spent more time outdoors, which could have exposed them to more outdoor asthma/wheeze triggers.

The contradictory effects observed for dietary vitamin D and serum 25(OH)D are not completely surprising since dietary vitamin D intake tends to be poorly correlated with serum vitamin levels. For instance, although we found that dietary vitamin D was a significant predictor of serum 25(OH) D ($p=0.03$), it only accounted for 4% of the variability in serum 25(OH)D levels, suggesting that non-dietary factors also influenced serum vitamin levels. In addition, it is possible that other dietary factors associated with vitamin D-rich foods were responsible for the observed effect. However, we examined possible food components, such as omega-3 fatty acids and calcium, and did not observe an association between dietary intakes and wheeze/asthma symptoms for these nutrients, although it remains possible that other, unevaluated food components play a role.

Another possible explanation of the discrepancy is the low DRI for dietary vitamin D (200 IU), which we used as our cut-off value for dietary adequacy. This level of intake is insufficient to raise serum concentrations to the level considered “optimal” by most vitamin D experts. Had we raised the dietary cut-off to 600 IUs—the new RDA level recently suggested by the Food and Nutrition Board – many more women would have had “suboptimal” intake, and the apparent risk associated with low intake may have been attenuated.

In addition to assessing the association between vitamin D status and wheeze/asthma, our study also identified the predictors of serum 25 (OH) D in this sample. In addition to total dietary vitamin D ($p=0.03$), intake of cold cereal ($p=0.02$) was also predictive of serum 25(OH)D levels. Among non-dietary factors, month of blood draw ($p=0.05$) and oral contraceptive use ($p<0.0001$) were positive predictors of serum 25(OH) D, and sunscreen use ($p=0.04$) was a negative predictor. Compared to other vitamin D-rich foods, such as tuna or milk, cold cereal was the strongest dietary predictor of serum vitamin D in our study, suggesting that vitamin D fortification is an important contributor to dietary intake. The associations between serum vitamin D and month of blood draw and sunscreen use are not surprising given the well-known major contribution of cutaneous synthesis to vitamin D status. In addition, we found that, after adjustment for covariates, oral contraceptive use was associated with 25(OH)D levels that were on average 24 nmol/L greater than those observed in women who did not use oral contraceptives. The positive association between oral contraceptive use and 25(OH)D levels has been described by others (Sowers et al., 1986; Harris and Dawson-Hughes, 1998); in fact, Harris and Dawson-Hughes (1998) reported the exact same increment in 25(OH)D levels—24 nmol/L—that we observed in our study. Whether oral contraceptive use actually improves vitamin D status is not known; however, other researchers have speculated that the association it may be due to an estrogen-triggered change in the relative proportions of free and protein-bound vitamin D (Harris and Dawson-Hughes, 1998).

## Strengths and Limitations

Our study has several important strengths. In addition to information on dietary vitamin D intake and supplement use, we also had a biomarker measure of vitamin D status. Most previous studies of asthma and vitamin D have only collected dietary

vitamin D data. Given the generally poor correlation between dietary vitamin D intake and serum levels, measuring serum concentration of 25(OH)D provides a more reliable method for determining vitamin D status since it reflects circulating levels, which include vitamin D derived both from diet and from cutaneous synthesis. In addition, our collection of questionnaire data concerning lifestyle factors, such as time spent outdoors and sunscreen use, helps to strengthen our analysis of predictors of 25(OH)D concentration. And finally, our cohort was quite homogenous, consisting only of young, healthy women with at least some college education. This homogeneity helps to reduce confounding; however, it limits the generalizability of our findings.

Our study also had some limitations. First, the sample size was relatively small (n=186). A larger sample size would have increased the statistical power to detect associations. Secondly, because the study was originally intended to assess the relationship between vitamin D status and premenstrual syndrome, women who routinely use corticosteroids were ineligible. Because steroid use is common among persons with more severe asthma, it is possible that we inadvertently excluded women with more pronounced symptoms. Exclusion of these women would tend to bias our results toward the null. Third, since nearly all participants were students on the UMass Amherst campus, study activities were suspended during the summer months when most students leave campus. It is possible that data collected during these high sun-exposure months, when serum 25(OH)D levels are likely to be substantially higher, may have altered our findings.

We found that dietary vitamin D intake less than 200 IU daily was associated with an increased risk of asthma/wheeze symptoms. Other dietary components in vitamin D-rich foods did not appear to influence asthma risk. In contrast, we found that the risk of

wheeze/asthma symptoms was actually lower among women with “suboptimal” serum concentrations of 25(OH)D compared to women with apparently “optimal” vitamin D status. We doubt that higher serum levels of 25(OIH)D are an actual risk factor for asthma. Rather, we speculate that women with higher serum vitamin D likely spent more time outdoors during months that support cutaneous vitamin D synthesis and thereby may have been exposed to allergens and other compounds that could trigger asthma/wheeze symptoms. Because of the small sample size of the current study and our inconsistent results, further studies using larger cohorts with more rigorous assessment of asthma symptoms are needed to confirm our observations.

# BIBLIOGRAPHY


Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab* 2002;4:80–90

Abu-Harb M, Bell F, Finn A, et al. IL-8 and neutrophil elastase levels in the respiratory tract of infants with RSV bronchiolitis. *Eur Respir J* 1999; 14:139-43.

Agerberth, B., J. Grunewald, E. Castanos-Velez, B. Olsson, H. Jörnvall, H. Wigzell, A. Eklund, and G. H. Gudmundsson. 1999. Antibacterial components in bronchoalveolar lavage fluid from healthy individuals and sarcoidosis patients. *Am. J. Respir. Crit. Care Med*. 160:283-290

Ayesha, I., Bala, T.S., Reddy, C.V., Raghuramulu, N. 2001. Vitamin D deficiency reduces insulin secretion and turnover in rats. *Diabetes Nutr. Metab*. 14:78-84.

Bals, R., X. Wang, M. Zasloff, and J. M. Wilson. 1998. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc. Natl. Acad. Sci. USA* 95:9541-9546

Bhalla AK, et al. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab.* 1983;57:1308–1310.

Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the Third National Health and Nutrition Examination Survey. *Chest* 2005;128:3792-8.

Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009;179:765-71

Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al.Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010;126:52-8

Boonstra A, et al. 1α,25-Dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells*. J Immunol*. 2001;167:4974–4980

Burns JS, Dockery DW, Speizer FE. Low levels of dietary vitamin D intake and pulmonary function in adolescents. *Proc Am Thoracic Soc* 2006;3:A526.

Busse WW. The role of leukotrienes in asthma and allergic rhinitis. *Clin Exp Allergy* 1996;26:868-79.

Busse WW, Lemanske RF,Jr. Asthma. *N Engl J Med* 2001;344:350-62.

Camargo CA, Rifas-Shiman SL, Litonjua AA et al. Prospective study of maternal intake of vitamin D during pregnancy and risk of wheezing illnesses in children at age 2 years. *J Allergy Clin Immunol* 2006

Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, et al. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at age 3 years. *Am JClin Nutr* 2007;85:788-95.

Camargo CA Jr, Ingham T, Wickens K, et al.: Cord blood 5-hydroxyvitamin D levels and risk of childhood wheeze in New Zealand [abstract]. *Am J Respir Crit Care Med* 2008, 177(Suppl):A993

Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997;7:439-43.

Chen TC, Holick MF. Vitamin D and prostate cancer prevention and treatment. *Trends Endocrinol Metab* 2003;14:423-30.

Ciaccio CE, Portnoy JM Vitamin D Insufficiency and Risk of Severe Asthma Exacerbations.*Curr Allergy Asthma R*ep. 2010 Aug 20

Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;22:789-815.

CDC. Asthma prevalence, health care use, and mortality, 2002. Hyattsville, MD: US Department of Health and Human Services, CDC, National Center for Health Statistics; 2004.

Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos Int* 2005;16:713-6.

Devereux G, Litonjua AA, Turner SW, Craig LCA, McNeill G, Martindale S, Helms PJ, Seaton A, Weiss ST. Maternal vitamin D intake during pregnancy and early childhood wheeze. *Am J Clin Nutr* 2007;85:853–859.

Dietary Reference Intakes for Calcium and Vitamin D (2010). National Academy Press: Washington, DC

Expert Panel Report 3  Nation Heart Lung and Blood Institution. http://www.nhlbi.nih.gov/health/dci/Diseases/Asthma/Asthma_WhatIs.html

Erkkola M, Kaila M, Nwaru BI, Kronberg-Kippilä C, Ahonen S, Nevalainen J, Veijola R, Pekkanen J, Ilonen J, Simell O, Knip M, Virtanen SM Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children *Clin Exp Allergy*. 2009 Jun;39(6):875-82

Fagan JK, Scheff PA, Hryhorczuk D, Ramakrishnan V, Ross M, Persky V. Prevalence of asthma and other allergic diseases in an adolescent population: association with gender and race . *Ann Allergy Asthma Immunol.* 2001 Feb;86(2):177-84.

Finucane KE, Greville HW, Brown PJ. Irreversible airflow obstruction. Evolution in asthma. *Med J Aust* 1985;142:602-4.

Fleer A, Krediet TG. Innate immunity: toll-like receptors and some more. A brief history, basic organization and relevance for the human newborn. *Neonatology* 2007;92:145-57.

Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin D .Washington (DC): National Academy Press; 1997.

Ford ES. The epidemiology of obesity and asthma. *J Allergy Clin Immunol* 2005;115:897,909; quiz 910.

Ginde AA, Mansbach JM, Camargo CA Jr. Vitamin D, respiratory infections, and asthma *Curr Allergy Asthma Rep*. 2009 Jan;9(1):81-7.

Gale C, Robinson S, Harvey N, Javaid M, Jiang B, Martyn C, et al. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr* 2007; Feb 21

Gern JE. Rhinovirus respiratory infections and asthma. *Am J Med* 2002;112 Suppl 6A:19S-27S.

Gern JE, Busse WW. Relationship of viral infections to wheezing illnesses and asthma. *Nat Rev Immunol* 2002;2:132-8.

Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Arch Intern Med* 2009;169:626-32

Gombart AF. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol* 2009;4:1151-65.

Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J* 2005;19:1067-77.

Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* 2004;158:531-7.

Hargreave FE, Ryan G, Thomson NC, et al. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981;68:347-55.

Harris SS, Dawson-Hughes B The Association of Oral Contraceptive Use with Plasma 25-hydroxyvitamin D Levels J Am CollNutr. 1998 Jun;17(3):282-4.

Heaney RP. Long-latency deficiency disease: insights from calcium and vitamin D. Am J Clin Nutr 2003;78:912-9.

Hewison M. Vitamin D and innate immunity. *Curr Opin Investig Drugs* 2008;9:485-90.

Holgate ST. Inflammatory and structural changes in the airways of patients with asthma. *Respir Med* 2000;94 Suppl D:S3-6.

Holgate ST. Genetic and environmental interaction in allergy and asthma. *J Allergy Clin Immunol* 1999a;104:1139-46.

Holgate ST. The epidemic of allergy and asthma. *Nature* 1999b;402:B2-4.

Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. *Mol Aspects Med* 2008a;29:361-8.

Holick MF. Diabetes and the vitamin D connection. *Curr Diab Rep* 2008b ;8:393-8.

Holick MF. Vitamin D and sunlight: strategies for cancer prevention and other health benefits. *Clin J Am Soc Nephrol* 2008c;3:1548-54.

Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-81.

Holick MF. Vitamin D: its role in cancer prevention and treatment. *Prog Biophys Mol Biol* 2006;92:49-59.

Holick MF. The vitamin D epidemic and its health consequences. *J Nutr* 2005;135:2739S-48S.

Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune disease, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004a;80(suppl):1678S-1688S.

Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004b;79:362-371.

Hypponen E, Sovio U, Wjst M, et al. Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. *Ann N Y Acad Sc*i 2004;1037:84-95.

Kinyamu HK, Gallagher JC, Knezetic JA, DeLuca HF, Prahl JM, Lanspa SJ. Effect of vitamin D receptor genotypes on calcium absorption, duodenal vitamin D receptor concentration, and serum 1,25 dihydroxyvitamin D levels in normal women. Calcif Tissue Int 1997;60:491-5.

Carbone L.D., Rosenberg E.W., Tolley E.A., Holick M.F.,Hughes M.F., Watsk M.A, Barrow K.D., Chen T.C., Wilkin N.K., Bhattacharya S.K., Dowdy J.C.,. Sayre R.M and Weber K.T., 25-Hydroxyvitamin D, cholesterol, and ultraviolet irradiation, *Metab. Clin. Exp.* 57 (2008), pp. 741–748

Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;111:450,63; quiz 464.

Lemire JM, et al. 1,25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity *in vitro*. *J Immunol. 1*985;134:3032–3035

Lemire JM, et al. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. *J Nutr.* 1995;125:1704S–1708S

Li, Y.C., Kong, J., Wei, M., Chen, Z.F., Liu, S.Q., and Cao, L.P. 2002. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 110:229-238.

Lips, P. (2006). Vitamin D physiology. *Pr Bio and Molr Bio*,92.4-8

Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;120:1031-5.

Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin R (March 2006). "Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response". *Science (journal)* 311 (5768): 1770–3.

Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III *Bone*. 2002 May;30(5):771-7

Martini LA, Wood RJ. Vitamin D status and the metabolic syndrome. *Nutr Rev* 2006;64:479-86

Matsioka LY, Ide L, Wortsman J, Maclaughlin JA and Holick MF Sunscreens Suppress Cutaneous Vitamin D3 Synthesis J Clin Endo & Meta Vol. 64, No. 6 1165-1168 doi:10.1210/jcem-64-6-1165

McConnell,R. Berhane,K.Gilliland,F. Islam,T. Gauderman,W.J.; London,S.J.; Avol,E.; Rappaport,E.B.; Margolis,H.G.; Peters,J.M. Indoor risk factors for asthma in a prospective study of adolescents *Epi*, 2002, 13, 3, 288-295,

Mohr SB, Garland CF, Gorham ED, Garland FC. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia* 2008;51:1391-8.

Moorman J E, Rudd RS et. al National Surveillance for Asthma---Untied States, 1980-2004 *MMWR Surveill Summ* 2007

National Health Interview Survey,Current Asthma Prevalence Percents by Age, United States, 2007

Norman, A. W. (2001). Vitamin D. In B. A. Bowman, & R. M. Russell (Eds.), Present knowledge in nutrition (8th ed., pp. 146). Washington, DC: ILSI.

Peden DB.Air pollution in asthma: effect of pollutants on airway inflammation *Ann Allergy Asthma Immunol*. 2001 Dec;87(6 Suppl 3):12-7

Pelaia G, Vatrella A, Gallelli L, Renda T, Cazzola M, Maselli R, Marsico SA. Respiratory infections and asthma *Respir Med*. 2006 May;100(5):775-84. Epub 2005 Nov 9

Pizzichini MM, Pizzichini E, Efthimiadis A, et al. Asthma and natural colds. Inflammatory indices in induced sputum: a feasibility study. *Am J Respir Crit Care Med* 1998;158:1178-84.

Platts-Mills TA, Woodfolk JA, Sporik RB. Con: the increase in asthma cannot be ascribed to cleanliness. *Am J Respir Crit Care Med* 2001;164:1107,8; discussion 1108-9.

Raviv S, Smith LJ. Diet and asthma. *Curr Opin Pulm Med* 2010;16:71-6.

Roberto J. Barrios, Farrah Kheradmand, La Keisha Batts, David B. Corry (2006) Asthma: Pathology and Pathophysiology. *Arcs Path & Lab Med*: April 2006, Vol. 130, No. 4, pp. 447-451.

Reisman J, Schachter HM, Dales RE, Tran K, Kourad K, Barnes D, Sampson M, Morrison A, Gaboury I, Blackman J Treating asthma with omega-3 fatty acids: where is the evidence? A systematic review. *BMC Complement Altern Med*. 2006 Jul 19;6:26.

Romieu I, Trenga C Diet and obstructive lung diseases *Epidemiol Rev.* 2001;23(2):268-87.

Rostand, S.G. 1997. Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension* 30:150-156.

Sentongo TA. Semaeo EJ, Stettler NS, et al. Vitamin D status in children, adolescents, and young adults with Crohn disease. *Am J Clin Nutr* 2002;76:1077-1081.

Shore SA. Obesity and asthma: possible mechanisms. *J Allergy Clin Immuno*l 2008;121:1087,93; quiz 1094-5.

Sowers M, Wallace RB, Hollis BW, Lemke JH: Parameters related to 25-OH-D levels in a population-based study of women. Am J Clin Nutr 43:621–628, 1986.
Stachan DP, Butland BK , et al Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. *BMJ* 1996: 312: 1195-1191

Stead WW, Senner JW, Reddick WT, Lofgren JP. Racial differences in susceptibility to infection by Mycobacterium tuberculosis. *N Engl J Med* 1990;322:422-7.

Sundeep S. Salvi, K. Suresh B, and Stephen TH Is Asthma Really Due to a Polarized T Cell Response Toward a Helper T Cell Type 2 Phenotype? *Am. J. Respir. Crit. Care Med*. 2001; 164: 1343-1346

Tanaka H, Abe E, Miyaura C, Kuribayashi T, Konno K, Nishii T and Suda T 1 alpha,25-Dihydroxycholecalciferol and a human myeloid leukaemia cell line (HL-60) *Biochem. J.* (1982) 204 (713–719

Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. *Am J Clin Nutr* 2003;77:1478-83.

Tavera-Mendoza LE, White JH. Cell defenses and the sunshine vitamin. *Sci Am* 2007;297:62,5, 68-70, 72

Thacher TD, Fischer PR, Strand MA, Pettifor JM. Nutritional rickets around the world: causes and future directions. *Ann Trop Paediatr* 2006;26:1-16.

Trump, D. L., Hershberger, P. A., Bernardi, R. J., Ahmed, S., Muindi, J., Fakih,M., et al. (2004). Anti-tumor activity of calcitriol: Pre-clinical and clinical studies. *J Steroid Biochem Mol Biol*, 89-90(1-5), 519-526.

Ulrik CS, Lange P. Cigarette smoking and asthma Monaldi Arch *Chest Dis*. 2001 Aug;56(4):349-53.

Van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol* 2005;97:93-101.

Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008;117:503-11.

Weber, G, Heilborn, JD, Chamorro Jimenez, CI, Hammarsjö, A, Törmä, H, Ståhle, M: Vitamin D induces the antimicrobial protein hCAP18 in human skin. *J Invest Dermatol* 2005 124: 1080–1082,

Weiss ST. Diet as a risk factor for asthma. *Ciba Found Symp* 1997;206:244,57: discussion 253-7.

Willett, W.C., Sampson, L., Stampfter, M.J., Rosner, B., Bain, C., Witschi, J.,Hennekens, C.H., & Speizer, F.E. (1985). Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am. J Epi*, 122(1), 51-65

Wjst M. The vitamin D slant on allergy. *Pediatr Allergy Immunol* 2006;17:477-83.

Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoidresistant asthma patients. *J Clin Invest* 2006;116:146-55.

Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). *J Cyst Fibros* 2007;6:403-10.